REVIEW ARTICLE

NOVEL INSITU POLYMERIC DRUG DELIVERY SYSTEM: A REVIEW

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ABSTRACT:

In situ forming polymeric formulations are drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation in situ, to form a gel. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, electrical sensitivity, enzyme sensitive from which the drug gets released in a sustained and controlled manner. Routes of administration are oral, ocular, rectal, vaginal, injectable and intraperitoneal. Various biodegradable polymers that are used for the formulation of in situ gels include gellan gum, alginic acid, xyloglucan, pectin, chitosan, poly (DL lactic acid), poly (DL-lactide-co-glycolide) and poly-caprolactone. The in situ gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems and good patient compliance, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. Evaluation of In situ gel systems include in vitro drug release studies, sol-gel transition temperature and gelling time, gel strength, viscosity & rheology, texture analysis, clarity. Commercial formulations of in situ polymeric systems are Regel Depot Technology, Cytoryn and Timoptic-Xe. Recent developments in the field of polymer science and technology has led to the development of various stimuli sensitive hydrogels like pH, temperature sensitive, which are used for the targeted delivery of proteins to colon, and chemotherapeutic agents to tumors. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing.

Keywords: Biodegradable polymers, polymeric gel, controlled release, in situ gels, poly (lactic-co-glycolic acid), sustained release.

INTRODUCTION:

Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems. Amongst the extensive research has been carried in designing of polymeric drug delivery systems. The development of in situ gel systems has received considerable attention over the past few years. In the past few years, increasing number of in situ gel forming systems have been investigated and many patents for their use in various biomedical applications including drug delivery have been reported. This interest has been sparked by the advantages shown by in situ forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort[1]. In situ gel formulations offers an interesting alternative for achieving systemic drug effects of parenteral routes, which can be inconvenient or oral route, which can result in unacceptably low bioavailability and passes the hepatic first-pass metabolism, in particular of proteins and peptides[2]. This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa, that problems generally encountered in semisolid dosage forms. In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange[3]. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulations. The advantages of using biodegradable polymers in clinical applications are apparent. Various natural and synthetic polymers are used for formulation development of in situ forming drug delivery systems[4]. This review attempts to discuss the newer developments and strategies for this drug delivery including physiological factors, physiochemical factors and formulation factors to be considered in the development of in-situ drug delivery system. Also, different types of smart polymers, their mechanisms of gel formation from the sol forms, evaluation and characterization of in situ polymeric formulations and commercial formulation are discussed.

IN SITU GELLING SYSTEM:-

This is a more desirable dosage form which can be deliver drug in solution form & create little to no problem of vision & frequently doses are not needed. This in situ gelling system is when exposed to physiological condition will shift to a gel phase. This new concept of production a gel in-situ was suggested first time in the early 1980s.Gelation occurs via the cross linking of polymer chain that can be achieved covalent bond formation (chemical cross linking) or non covalent bond formation (physical cross linking)[5]. This system described as low viscosity solution that undergoes phase transition in conjuctival cul-de-sac to form viscoelestic gel due to conformational changes of polymer in response to physiological environment[6]. The rate of in situ gel formation is important because between instillation in eye & before a strong gel is formed; the solution or weak gel is produced by the fluid mechanism of eye[7].

IMPORTANCE OF IN SITU GELLING SYSTEM:-

- The major importance is the possibilities of administrating accurate &reproducible quantities compared to already formed gel[8].
- In-situ forming polymeric delivery system such as ease of administration &reduced frequency of administration improved patient compliance & comfort[9].
- Poor bioavailability & therapeutic response exhibited by conventional ophthalmic solution due to rapid precorneal elimination of drug may be overcome by use of gel system that are instilled as drops into eye &undergoes a sol-gel transition from instilled dose[10].
- Liquid dosage form that can sustain drug release & remain in contact with cornea of eye for extended period of time is ideal[11].
- Reduced systemic absorption of drug drained through the nasolacrimal duct may result in some undesirable side effects[12, 13].

IDEAL CHARACTERISTICS OF POLYMERS[14]:-

A polymer used to in situ gels should have following characteristics-

- It should be biocompatible.
- It should be capable of adherence to mucus.
- It should have pseudo plastic behaviour.
- It should be good tolerance & optical activity.
- It should influence the tear behaviour.
- The polymer should be capable of decrease the viscosity with increasing shear rate there by offering lowered viscosity during blinking & stability of the tear film during fixation.

The first use of gel for medical preparation was represented by Wichterle & Lim in 1960 in manufacturing of soft contact lenses & implant material from Hydroxyethyl Methacrylate polymer[15].

POLYMERS USED IN IN SITU GELLING SYSTEM

Gellangum

Gellan gum (commercially available as Gelrite TM or Kelcogel TM) is an anionic deacetylated exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucuronic acid residues[16]. It has the tendency of gelation which is temperature dependent or cations induced. This gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water[17]. The formulation consisted of gellan solution with calcium chloride and sodium citrate complex. When administered orally, the calcium ions are released in acidic environment of stomach leading to gelation of gellan thus forming a gel in situ[18]. In situ gelling gellan formulation as vehicle for oral delivery of theophylline is reported.

Xyloglucan

Xyloglucan is a polysaccharide derived from tamarind seeds and is composed of a (1-4)-β-D-glucan backbone chain, which has (1-6)-α-D xylose branches that are partially substituted by (1-2)-β-D-galactoxylose[19]. When xyloglucan is partially degraded by β- galactosidase, the resultant product exhibits thermally reversible gelation by the lateral stacking of the rod like chains. The sol-gel transition temperature varies with the degree of galactose elimination. It forms thermally reversible gels on warming to body temperature. Its potential application in oral delivery exploits the proposed slow gelation time (several minutes) that would allow in-situ gelation in the stomach following the oral administration of chilled xyloglucan solution[20]. Xyloglucan gels have potentially been used for oral, intraperitoneal, ocular and rectal drug delivery[21, 22].

Alginic acid

Alginic acid is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1, 4-glycosidic linkages. The proportion of each block and the arrangement of blocks along the molecule vary depending on the algal source. Dilute aqueous solutions of alginates form firm gels on addition of di and trivalent metal ions by a cooperative process involving consecutive glucuronic residues in the α-Lglucuronic acid blocks of the alginate chain[23]. Alginic acid can be chosen as a vehicle for ophthalmic formulations, since it exhibits favorable biological properties such as biodegradability and nontoxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability to gel in the eye, but also because of its mucoadhesive properties[24].

Xanthum gum

Xanthan gum is a high molecular weight extra cellular polysaccharide produced by the fermentation of the gram-negative bacterium Xanthomonas campestris. The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β- D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronic acid-α-D-mannose attached with alternate glucose residues of the main chain. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain[25].

Chitosan

Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shell. Chitosan is a biocompatible pH dependent cationic polymer, which remains dissolved in aqueous solutions up to a pH of 6.2[26]. Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel like precipitate. The pH gelling cationic polysaccharides solution are transformed into thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking by addition of polyol salts bearing a single anionic head such as glycerol, sorbitol, fructose or glucose phosphate salts to chitosan aqueous solution[27].

Carbopol

Carbopol is a well known pH dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. HPMC is used in combination with carbopol to impart the viscosity to carbopol solution, while reducing the acidity of the solution. Various water

soluble polymers such as carbopol system-hydroxypropylmethylcellulose system, poly (methacrylic acid)-poly (ethylene glycol) come under the category of pH-induced in-situ precipitating polymeric systems. Based on this concept, the formulation and evaluation of an ophthalmic delivery system for indomethacin for the treatment of uveitis was carried out. A sustained release of indomethacin was observed for a period of 8 h in vitro thus considering this system as an excellent candidate for ocular delivery. A pH induced in-situ precipitating polymeric system (an aqueous solution of carbopol-HPMC system) was designed and developed by Ismail et al. for plasmid DNA delivery[28].

Pectin

Pectins are a family of polysaccharides, in which the polymer backbone mainly comprises α-(1-4) D galacturonic acid residues. Low methoxy pectins (degree of esterification <50%) readily form gels in aqueous solution in the presence of free calcium ions, which crosslink the galacturonic acid chains in a manner described by egg-box model. Although the gelation of pectin will occur in the presence of H + ions, a source of divalent ions, generally calcium ions is required to produce the gels that are suitable as vehicles for drug delivery[29]. The main advantage of using pectin for these formulations is that it is water soluble, so organic solvents are not necessary in the formulation. Divalent cations present in the stomach, carry out the transition of pectin to gel state when it is administered orally. Calcium ions in the complexed form may be included in the formulation for the induction of pectin gelation[30]. Sodium citrate may be added to the pectin solution to form a complex with most of calcium ions added in the formulation. By this means, the formulation may be maintained in a fluid state (sol), until the breakdown of the complex in the acidic environment of the stomach, where release of calcium ions causes gelation to occur. The quantities of calcium and citrate ions may be optimized to maintain the fluidity of the formulation before administration and resulting in gelation, when the formulation is administered in stomach. The potential of an orally administered in situ gelling pectin formulation for the sustained delivery of Paracetamol has been reported[4].

Pluronic F-127

Poloxamers or pluronic (marketed by BASF Corporation) are the series of commercially available difunctional triblock copolymers of non-ionic nature. They comprise of a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly ethylene oxide[31]. Due to the PEO/PPO ration of 2:1, when these molecules are immersed into the aqueous solvents, they form micellar structures above critical micellar concentration. They are regarded as PEO-PPO-PEO copolymers. Chemically they are Oxirane, methyl-, polymer with oxirane or α-Hydro-ω- hydroxypoly (oxyethylene) a poly (oxypropylene) b poly (oxyethylene) a block copolymer. The pluronic triblock copolymers are available in various grades differing in molecular weights and physical forms. Depending upon the physical designation for the grades are assigned, as F for flakes, P for paste, L for liquid. Pluronics or Poloxamers also undergo in situ gelation by temperature change. They are triblock copolymers consisting of poly (oxyethylene) and poly (oxypropylene) units that undergo changes in solubility with change in environment temperature. Pluronic ™ F 127. A 25-40% aqueous solution of this material will gel at about body temperature, and drug release from such a gel occurs over a period of up to one week[32]. Pluronic F-127 was used as an in situ gel forming polymer together with mucoadhesive polymers such as Carbopol 934 and hydroxylpropylmethylcellulose to ensure long residence time at the application site. Controlled release of drug was achieved in-vitro indicating antimycotic efficacy of developed formulation for a longer period of time[33].

Synthetic polymers

Synthetic polymers are popular choice mainly for parenteral preparations. The trend in drug delivery technology has been towards biodegradable polymers, requiring no follow up surgical removal, once the drug supply is depleted. Aliphatic polyesters such as poly (lactic acid), poly (glycolic acid), poly (lactide- coglycolide), poly (decalactone), poly ε-caprolactone have been the subject of the most extensive recent investigations. Synthetic polymers are popular choice mainly for parenteral preparations. The trend in drug delivery technology has been towards biodegradable polymers, requiring no follow up surgical removal, once the drug supply is depleted. Aliphatic polyesters such as poly (lactic acid), poly (glycolic acid), poly (lactide- coglycolide), poly (decalactone), poly ε-caprolactone have been the subject of the most extensive recent investigations[26]. Various other polymers like triblock polymer systems composed of poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly(DL-lactide), blends of low molecular weight poly(D,L-lactide) and poly(ε- caprolactone) are also in use. These polymers are mainly used for the injectable in situ formulations. The feasibility of lactide/glycolide polymers as excipients for the controlled release of bioactive agents is well proven. These materials have been subjected to extensive animal and human trials without evidence of any harmful side effects. When properly prepared under GMP conditions from purified monomers, the polymers exhibit no evidence of inflammatory response or other adverse effects upon implantation[34]. Another type of synthetic polymeric system includes the in situ cross linked system, where the polymers form cross linked networks by means of free radical reactions that may occur by means of light (photopolymerizable systems) or heat(thermosetting systems).

Thermosetting systems are in the sol form when initially constituted, but upon heating, they set into their final shape. This sol-gel transition is known as curing. But if this cured polymer is heated further, it may lead to degradation of the polymer. Curing mainly involves the formation of covalent cross links between polymer chains to form a macromolecular network. Dunn et al. designed a thermosetting system using biodegradable copolymers of DL-lactide or L-lactide with ε-caprolactone for prosthetic implant and slow release drug delivery systems. This system is liquid outside the body and is capable of being injected by a syringe and needle and once inside the body, it gels. In in situ precipitating polymeric systems, the polymer precipitation from solution may lead to gel formation in situ and this precipitation can be induced by

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change in temperature (thermosensitive systems), solvent removal or by change in pH[35]. An important example of thermosensitive polymer is poly-(N-isopropyl acrylamide), [poly (NIPAAM)], which is used for the formation of in situ gels. It has lower critical solution temperature phase separation at about 32. The polymers such as poly (DL-lactide), poly (DL-lactide-co-glycolide) and poly (DL-lactide-co-Ξ-caprolactone) form solvent-removal precipitating polymeric systems[36].

APPROACHES OF IN SITU GEL DRUG DELIVERY

There are four broadly defined mechanisms used for triggering the in situ gel formation of biomaterials: Physiological stimuli (e.g., temperature and pH), physical changes in biomaterials (e.g., solvent exchange and swelling), chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization).

In situ formation based on physiological stimuli:

Temperature triggered system–

Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research[37]. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is faccilated and no external source of heat other than that of body is required for trigger gelation. A useful system should be tailorable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity. Three main strategies are exists in engineering of thermoresponsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels (1, 3).

Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical temperature (LCST) transition between ambient and physiologic temperature is used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (Nisopropylacrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which result on precipitation of PNIPAAm from the solution at the LCST. Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPOPEO) triblock co-polymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequences of a disorder-order transition in micelle packing which makes these polymers suitable for in situ gelation[38]. A positive temperaturesensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly (acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling[39]. The most commonly used thermoreversible gels are these prepared from poly (ethylene oxide)-b-poly (propylene oxide)-b poly (ethylene oxide) (Pluronics®, Tetronics®, poloxamer). Polymer solution is a free flowing liquid at ambient temperature and gels at body temperature[40]. Cappello et al. developed novel "protein polymers" ProLastins, which undergo an irreversible sol gel transition. When injected as a solution into the body, the material forms a firm, stable gel within minutes. It remains at the site of injection providing absorption times from less than one week to many months. Such a system would be easy to administer into desired body cavity[41].

pH triggered systems -

Another formation of in situ gel based on physiologic stimuli is formation of gel is induced by pH changes[37]. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH .The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups[39]. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives[42]. Likewise polyvinylacetal diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition[43]. Drug formulated in liquid solutions have several limitations, including limited bioavailability and propensity to be easily removed by tear fluid. Kumar and Himmelstein sought to minimize this factor and maximize this drug delivery by making a poly (acrylic acid) (PAA) solution that would be gel at pH 7.4.The author found that at concentrations high enough to cause gelation, however, the low pH of PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH responsive polymer mixtures that was sol at pH 4 and gel at pH 7.4[44]. Mixtures of poly (methacrylic acid) (PMA) and poly (ethylene glycol)

(PEG) also has been used as a pH sensitive system to achieve gelation[45].

In situ formation based on physical mechanism-

Swelling

In situ formation may also occur when material absorbs water from surrounding environment and expand to occur desired space[46]. One such substance is myverol 18-99 (glycerol mono-oleate), which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some Bioadhesive properties and can be degraded in vivo by enzymatic action[47].

Diffusion

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. Nmethyl pyrrolidone (NMP) has been shown to be useful solvent for such system[48].

In situ formation based on chemical reactions

Chemical reactions that results in situ gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.

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Ionic crosslinking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones[49]. While k-carrageenan forms rigid, brittle gels in reply of small amount of K+, i-carrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes in situ gelling in the presence of mono- and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low-methoxy pectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca2+ due to the interaction with glucuronic acid block in alginate chains[50].

Enzymatic cross-linking

In situ formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation[51].

Photo-polymerisation-

Photo-polymerisation is commonly used for in situ formation of biomaterials. A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel[37]. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo-polymerisation in the presence of suitable photo- initiator. Typically long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is not used often because it has limited penetration of tissue and biologically harmful. A ketone, such as 2, 2 dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo-polymerization, where as camphor quinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence in vivo[52]. Photopolymerizable systems when introduced to the desired site via injection get photo cured in situ with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation. A photopolymerizable, biodegradable hydrogel as a tissue contacting material and controlled release carrier is reported by Sawhney et al[53].

FORMULATION DESIGN

The design of in-situ gel formulation depends on the physicochemical properties of the drug molecule, the diseased condition for which treatment is required, the patient population and the marketing preference. Physico-chemical factors include molecular weight, lipophilicity and molecular charge; an anatomical and physiological factor includes membrane transport, pH of tissue fluid, and mucocilliary clearance (as in case of nasal administrations). While formulation factors include clarity, pH, gelation temperature, viscosity, osmolarity, spreadability[3].

APPLICABILITY OF IN SITU POLYMERIC DRUG DELIVERY

SYSTEM:-

Oral drug delivery system:-

The pH-sensitive hydrogels have a potential use in site-specific delivery of drugs to specific regions of the GI tract. Hydrogels made of varying proportions of PAA derivatives and crosslinked PEG allowed preparing silicone microspheres, which released prednisolone in the gastric medium or showed gastroprotective property[54]. Cross-linked dextran hydrogels with a faster swelling under high pH conditions, likewise other polysaccharides such as amide pectins, guar gum and insulin were investigated in order to develop a potential colon-specific drug delivery system. W. Kubo et al.[55] developed the formulations of gellan and sodium alginate both containing complexed calcium ions that undergo gelation by releasing of these ions in the acidic environment of the stomach. Oral delivery of paracetamol was studied.

For the oral in situ gel delivery system pectin, xyloglucan & gellan gum natural polymers are used. Pectin formulation for sustained delivery of paracetamol has been reported[56]. Advantages of pectin is water soluble so, no need to add organic solvent.

Ocular drug delivery system:-

In ocular delivery system natural polymers like gallan gum, alginic acid & xyloglucan are most commonly used. For local ophthalmic delivery system various compounds like antimicrobial agent, anti-inflammatory agent & autonomic drugs are used to relieve intra ocular tension in glaucoma. Conventional delivery system often result in poor availability & therapeutic response because high tear fluid turn over & dynamics which cause rapid elimination of the drug from the eye so, the overcome the bioavailability problem ophthalmic in-situ gel was developed[57].

To improve the bioavailability viscosity enhancers such as Hydroxy Propyl Methyl Cellulose, Carboxy Methyl Cellulose, Carbomers, Poly Vinyl alcohol used to increase the viscosity of formulation in order to prolong the precorneal residence time & improve the bioavailability, ease to manufacture. Penetration enhancer such as preservatives, chelating agent, surfactants are used to enhance corneal drug penetration[58].

Nasal drug delivery system:-

In nasal in-situ gel system gallan gum & xanthan gum are used as in-situ gel forming polymers Momethasone furoate

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was evaluated for it's efficacy for the treatment of allergic rhinitis[59]. Animal study were conducted using allergic rhinitis model & effect of in-situ gel on antigen induced nasal symptoms in sensitizes rats was observed. In-situ gel was found to inhibit the increase in nasal symptoms are compared to marketed preparation nosonex (Momethasone furoate suspension 0.05%).

Rectal drug delivery system:-

The rectal route may be used to deliver many types of drugs that are formulated as liquid, semisolid (ointments, creams and foams) and solid dosage forms (suppositories). Conventional suppositories often cause discomfort during insertion. In addition, suppositories are unable to be sufficiently retained at a specific position in the rectum, sometimes they can migrate up-wards to the colon that makes them possible for drug to undergo the first-pass effect. Choi et al.[60] developed novel in situ gelling liquid suppositories with gelation temperature at 30–36°C. Poloxamer 407 and/ or poloxamer 188 were used to confer the temperature-sensitive gelation property.

In-situ gel possesses a potential application for rectal & vaginal route .Miyazaki et al. investigated the use of xyloglucan based thermo reversible gel for rectal drug delivery of Indomethacin. Administration of indomethacin loaded xyloglucan based system to rabbit indicated broad drug absorption & a longer drug residence time as compared to that resulting after administration of commercial suppository. For better therapeutic efficacy & patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating Clotrimazole-β-cyclodextrin complex formulated for treatment of vaginitis[61].

Vaginal drug delivery system:-

The vagina, in addition to being an important organ of reproductive tract, serves as a potential route for drug administration. Formulations based on a thermo-plastic graft copolymer that undergo in situ gelation have been developed to provide the prolonged release of active ingredients such as nonoxynol-9, progestins, estrogens, peptides and proteins[62]. Chang et al.[63] have recently reported a mucoadhesive thermo-sensitive gel (combination of poloxamers and polycarbophil), which exhibited, increased and prolonged antifungal activity of clotrimazole in comparison with conventional PEG-based formulation.

Injectable drug delivery system:-

One of the most obvious ways to provide sustained-release medication is to place the drug in delivery system and inject or implant the system into the body tissue. Thermoreversible gels mainly prepared from poloxamers are predominantly used[64]. The suitability of poloxamer gel alone or with the addition of hydroxypropylmethylcellulose (HPMC), sodium carboxymethylcellulose (CMC) or dextran was studied for epidural administration of drugs in vitro[65]. The compact gel depot acted as the rate limiting step and significantly prolonged the dural permeation of drugs in comparison with control solutions. J. M. Barichello et al.[66] evaluated Pluronic F127 gels, which contained either insulin or insulin-PLGA nanoparticles with conclusion, that these formulations could be useful for the preparation of a controlled delivery system. Likewise, poloxamer gels were tested for intramuscular and subcutaneous administration of human growth hormone[67] or with the aim to develop a long acting single dose injection of lidocaine[64]. J. R. DesNoyer and A. J. McHugh[68] invented a new class of injectable controlled release depots of protein which consisted of blends of Pluronics with poly (D, L-lactide)/1-methyl-2- pyrrolidone solutions. Some other thermosensitive hydrogels may also be used for parenteral administration. ReGel ® (triblock copolymer PLGAPEG-PLGA) was used as a drug delivery carrier for the continuous release of human insulin[69]. Steady amounts of insulin secretion from the Re- Gel ® formulations up to day 15 of the subcutaneous injections were achieved. B. Jeong et al.[70] reported the synthesis of a biodegradable poly (ethylene oxide) and poly (L-lactic acid) hydrogel, which exists in a form of sol at an elevated temperature (around 45°C) and forms a gel after subcutaneous injection and subsequent rapid cooling to body temperature. In-situ forming Injectable drug delivery system, cross linking of hydrazide modified by aluronic acid with aldehyde modified version of cellulose derivatives such as carboxy methyl cellulose, methyl cellulose, hydroxy propyl methyl cellulose are used. These in-situ forming gel were used for preventing postoperative peritoneal adhesion thus avoiding pelvic pain, bowel obstruction & infertility. For a better therapeutic efficacy & patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating Clotrimazole-β-cyclodextrin complex was formulated for treatment of virginities[71].

Dermal and transdermal drug delivery system:-

Thermally reversible gel of Pluronic F127 was evaluated as vehicle for the percutaneous administration of Indomethacin[72]. In-vivo studies suggest that 20% w/w aqueous gel may be of practical use as a base for topical administration of the drug. Poloxamer 407 gel was found suitable for transdermal delivery of insulin[73]. The combination of chemical enhancers and iontophoresis resulted in synergistic enhancement of insulin permeation.

EVALUATION AND CHARACTERIZATION OF IN SITU GELLING SYSTEM:-

In-situ gel evaluated & characterized by the following parameters-

Clarity:-

The clarity of formulated solution is determined by visual inspection under black & white Background[74].

Texture analysis:-

The consistency, firmness &cohesiveness of in situ gel are assessed by using texture profile analyzer which mainly indicated gel strength & easiness in administration in vivo higher value of adhesiveness of gel are needed to maintain an intimate contact with mucus surface[75].

pH of gel:-

pH can be determined formulation is taken in beaker & 1ml NaOH added drop wise with continuous stirring. pH is checked by using pH meter[76].

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Sol-Gel transition temperature and gelling time

For in situ gel forming systems incorporating thermoreversible polymers, the sol-gel transition temperature may be defined as that temperature at which the phase transition of sol meniscus is first noted when kept in a sample tube at a specific temperature and then heated at a specified rate. Gel formation is indicated by a lack of movement of meniscus on tilting the tube. Gelling time is the time for first detection of gelation as defined above[19].

Gel-Strength

This parameter can be evaluated using a rheometer. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the sol form. This gel containing beaker is raised at a certain rate, so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface[19].

Gelling capacity:-

In-situ gel is mix with simulated tear fluid (in the proportion of 25:7 i.e. application volume 25µl & normal volume of tear fluid in eye is 7µl) to find out gelling capacity of ophthalmic product. The gelation assessed visually by noting the time for & time taken for dissolution of the formed gel[77].

Rheological studies:-

The viscosity measured by using Brookfield viscometer, cone & plate viscometer. In-situ gel formulation is placed in sample tube. Formulation should have viscosity 5-1000 mPas, before gelling & after ion gel activation by eye will have viscosity of from about 50-50,000 mPas[77, 78].

Isotonicity evaluation:-

Isotonicity is important characteristics of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparation are subjected to isotonicity testing, science they exhibited good release characteristics & gelling capacity & the requite velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic formulation[79].

Swelling studies:-

Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature .The cell contains artificial tear fluid (composition – 0.67g Nacl, 0.20g NaHCo3, 0.008g Cacl2.2H2o & distilled water q.s to 100g)[80]. swelling medium equilibrating at 370c one milliliter of formulated solution is placed in dialysis bag & put into the swelling medium. At specific time interval the bag is removed from the medium & weight is recorded. The swelling of the polymer gel as a function of time is determined by using the following relationship[81, 82].

$$\% St = (Wt - W_0)\ 100/W_0$$

Where,

St = Swelling at time‘t’.

W_0=Initial weight of gelling solution.

Wt=Final weight of gel.

Statistical analysis:-

Analysis of variance (ANOVA) is used the testing the difference between calculated parameters using SPSS statistical package. Statistical difference yielding $P \leq 0.05$ is considered[83]. Duncan multiple comparison is applied when necessary to identify which of the individual formulations are significantly different[84].

High performance liquid chromatography:-

The HPLC system is used in reversed phase mode. Analysis is performed on a Nova pack C18 packed column (150 mm length X 3.9 mm i.d)[85].

Fourier transformer infra red:-

The possibility of drug excipient interaction is investigated by FTIR studies. The FTIR graph of pure drug & combination of drug with excipient are recorded by using KBR pellets[85, 86].

Thermal analysis:-

Thermo gravimetric analysis can be conducted for in situ forming polymeric system to quantitative the percentage of water in hydrogel. Different scanning calorimetry is used to observed, if there are many changes in thermograms as compared with pure ingredients used thus indicating the interaction[87].

In vitro drug release studies:-

In vitro release study of in situ gel solution is carried out by using Franz diffusion cell. The formulation is placed in donor compartment & freshly prepared simulated tear fluid in receptor compartment. Between receptor & donor compartment dialysis membrane is placed (0.22 µm pore size). The whole assembly is placed on thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37ºc± 0.5ºc.

1ml sample is withdrawn at predetermined time interval of 1hr for 6hrs the sample volume of fresh medium is replaced. The withdrawn sample is diluted to 10ml in volumetric flask with respective solvent & analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers peppas & Fickinian diffusion mechanism of their kinetics[88].

Ocular irritancy studies:-

Ocular irritancy studies are performed on male albino rabbits, weighing 1-2 kg. The modified Draize technique is used for ocular irritation potential of ophthalmic products[89]. The formulation is placed in lower cul-de-sac & irritancy is tested at time interval of 1hr, 2hrs, 48hrs, 72hrs, & 1 week after administration[90]. The rabbits are observed periodically for redness, swelling, & watering of eyes[91].

Antimicrobial activity:-

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Antimicrobial efficacy studies are carried out to ascertain the biological activity of sol-gel-system against microorganisms. This is determined in agar diffusion medium employing 'Cup Plate Techniques'[92]. The microbial growth of bacteria is measured by conc. Of antibiotic & compared with that produced by known conc. Of standard preparation of antibiotic & carried out the microbial assay serial dilution method is employed[93, 94].

Sterility testing:-

Sterility testing is carried out as per the IP 1996. The formulation is incubating for not less than 14 days at 300-350c in the fluid thioglycolate medium to find the growth of bacteria & at 200-250 c in Soya bean casein digest medium to find the growth of fungi in formulation[95].

Accelerated stability studies:-

Formulation is replaced in amber colored vials & sealed with aluminum foil for the short term accelerated stability study at 40± 20 c & 75 ±5% RH as per International Conference of Harmonization (ICH) State Guidelines. Sample is analyzed at every month for clarity, pH, gelling capacity, drug content, rheological evaluation & in vitro dissolution[96].

Histopathological studies

Two mucosa tissue pieces (3 cm2) were mounted on in vitro diffusion cells. One mucosa was used as control (0.6 mL water) and the other was processed with 0.6 mL of optimized organogel (conditions similar to in vitro diffusion). The mucosa tissues were fixed in 10% neutral carbonate formalin (24 hours), and the vertical sections were dehydrated using graded solutions of ethanol. The subdivided tissues were stained with hematoxylin and eosin. The sections under microscope were photographed at original magnification ×100. The microscopic observations indicate that the organogel has no significant effect on the microscopic structure of the mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultrastructure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged[97].

COMMERCIAL FORMULATIONS OF IN-SITU POLYMERIC SYSTEMS AT A GLANCE

Regel: depot-technology

Regel is one of the Macromed's proprietary drug delivery system and based on triblock copolymer, composed of poly (lactide-co-glycolide)-poly (ethylene glycol)-poly (lactide-co-glycolide). It is a family of thermally reversible gelling polymers developed for parenteral delivery that offers a range of gelation temperature, degradation rates and release characteristics as a function of molecular weight, degree of hydrophobicity and polymer concentration. Following injection, the physical properties of polymer undergo a reversible phase change resulting in formation of a water insoluble, biodegradable gel depot. Oncogel is a frozen formulation of paclitaxel in Regel. It is a free flowing liquid below room temperature which upon injection forms a gel in-situ in response to body temperature. hGHD-1 is a novel injectable depot formulation of human growth hormone (hGH) utilizing Macromed's Regel drug delivery system for treatment of patients with hGHdeficiency[98].

Cytoryn

This is one of the Macromed's products, which is a novel, peritumoral, injectable depot formulation of interleukin-2 (IL-2) for cancer immunotherapy using Regel drug delivery system. It is a free flowing liquid below room temperature that instantly forms a gel depot upon injection from which the drug is released in a controlled manner. Cytoryn enhances the immunological response by safely delivering four times the maximum tolerated dose allowed by conventional IL-2 therapy. Cytoryn also activates the systemic antitumor immunity. Regel system stabilizes and releases IL-2 in its bioactive form. The release of drugs is controlled by the rate of diffusion from and degradation of the depot.

Timoptic-XE

It is a timolol maleate ophthalmic gel formulation of Merck and Co. Inc., supplied as a sterile, isotonic, buffered, aqueous gel forming solution of timolol maleate. This formulation is available in two dosage strengths 0.25% and 0.5% in market. The pH of the solution is approximately 7.0, and the osmolarity is 260-330 mOsm. Each ml of Timoptic-XE 0.25% contains 2.5 mg of timolol (3.4 mg of timolol maleate). Inactive ingredients include gellan gum, tromethamine, mannitol, and water for injection and the preservative used is benzododecinium bromide 0.012%. Timoptic- XE, when applied topically on the eye, reduces the elevated, as well as normal intraocular pressure, whether or not accompanied by glaucoma.

CONCLUSION

In conclusion, the primary requirement of a successful controlled release product focuses on increasing patient compliance which the *in situ* gel offers. Exploitation of polymeric in- situ gel for controlled release of various drugs provides a number of advantages over conventional dosage forms. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems.

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